

Figure S1

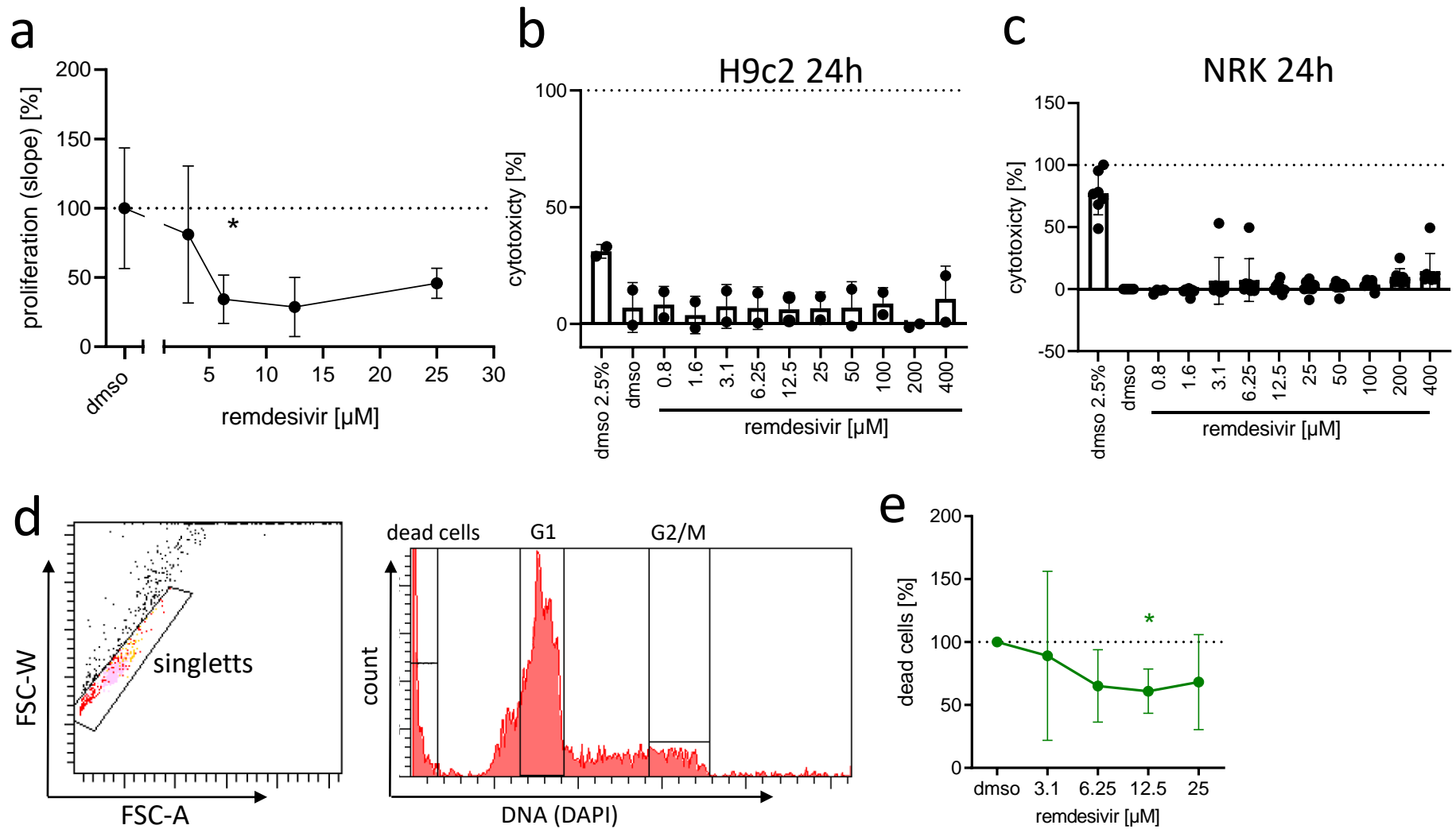


Fig. S1 The effect of remdesivir on growth and viability of H9c2 and NRK-52E cells
(a) H9c2 cells were treated with increasing concentrations of remdesivir 2 h after seeding. The proliferation-slope of H9c2 cells was derived from increasing well coverage values over 86 h. Data were normalized to the mean of solvent controls (dmso = 100 %, dashed line). N = 4-6. RM one-way ANOVA, Dunnett's multiple comparisons test. **(b)** H9c2 (n = 2) or **(c)** NRK-52E (n = 7-8) were treated with increasing concentrations of remdesivir for 24 h and LDH-activity was measured in the supernatants (LDH-activity after 10-20 % dmso-treatment was defined as 100 % cytotoxicity). **(d)** FACS gating strategy in NRK-52E cells for single dead cells and cells in G1 or G2/M phase. **(e)** Quantification of the frequency of dead cells in NRK-52E cells treated with remdesivir for 24 h measured by FACS (n = 6; Kruskal-Wallis-test with Dunn's multiple comparisons test) * p < 0.05, ** p < 0.01, *** p < 0.001

Figure S2

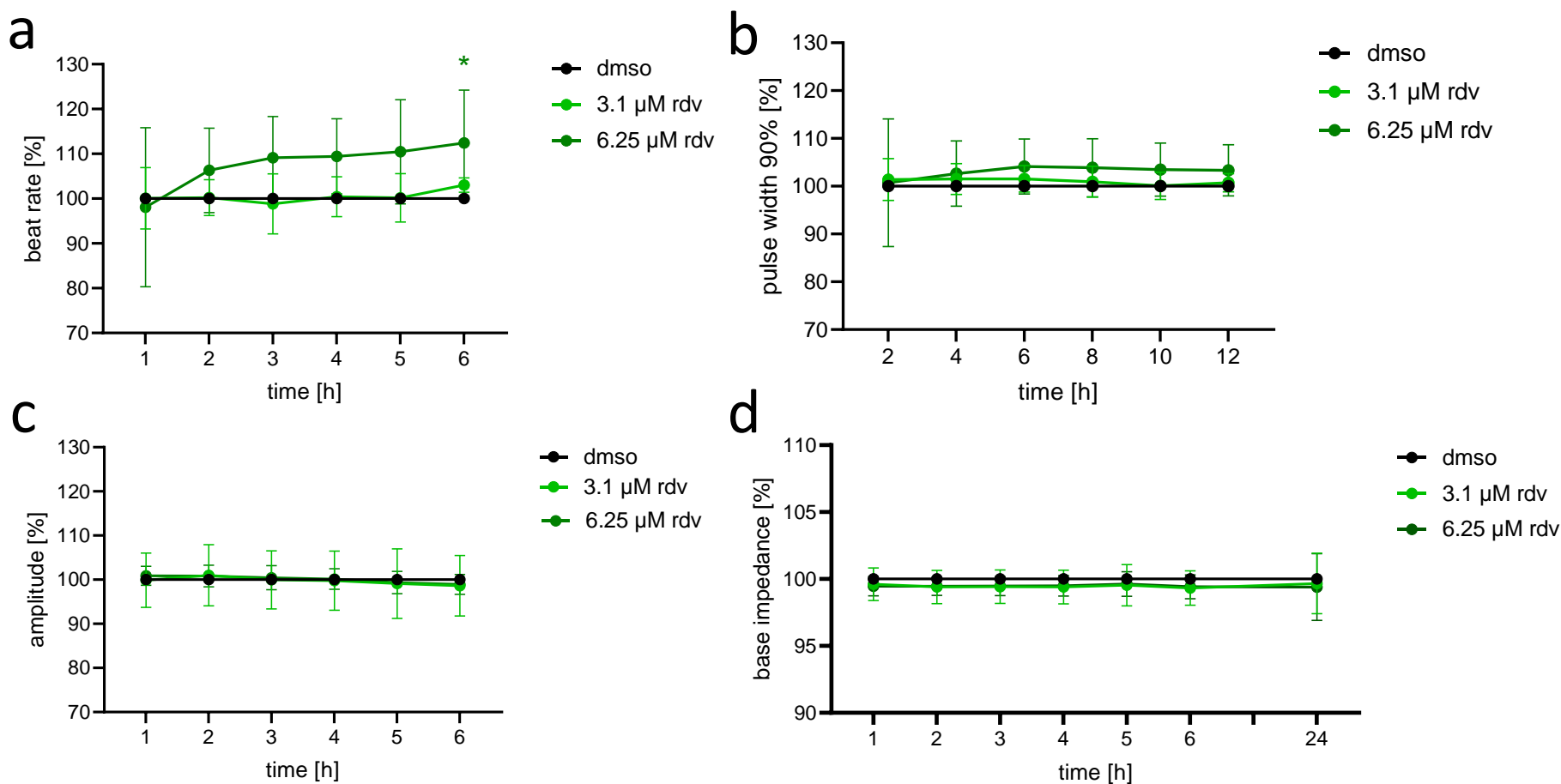


Fig. S2 Beating behaviour of NMCM changed by late treatment with remdesivir
NMCM were treated with 3.1 or 6.25 μ M of remdesivir or with solvent control (dmso). The parameters beat rate (a), pulse width 90 % (b), beat amplitude (c) and base impedance (d) were calculated from 20 sec. impedance recordings every hour after treatment. Data were normalized to solvent control (dmso = 100 %) in each experiment, n = 4-7 independent experiments run with 4-6 technical replicates (wells) each. Two-way ANOVA, Dunnett's multiple comparisons test * p < 0.05, ** p < 0.01, *** p < 0.001

Figure S3

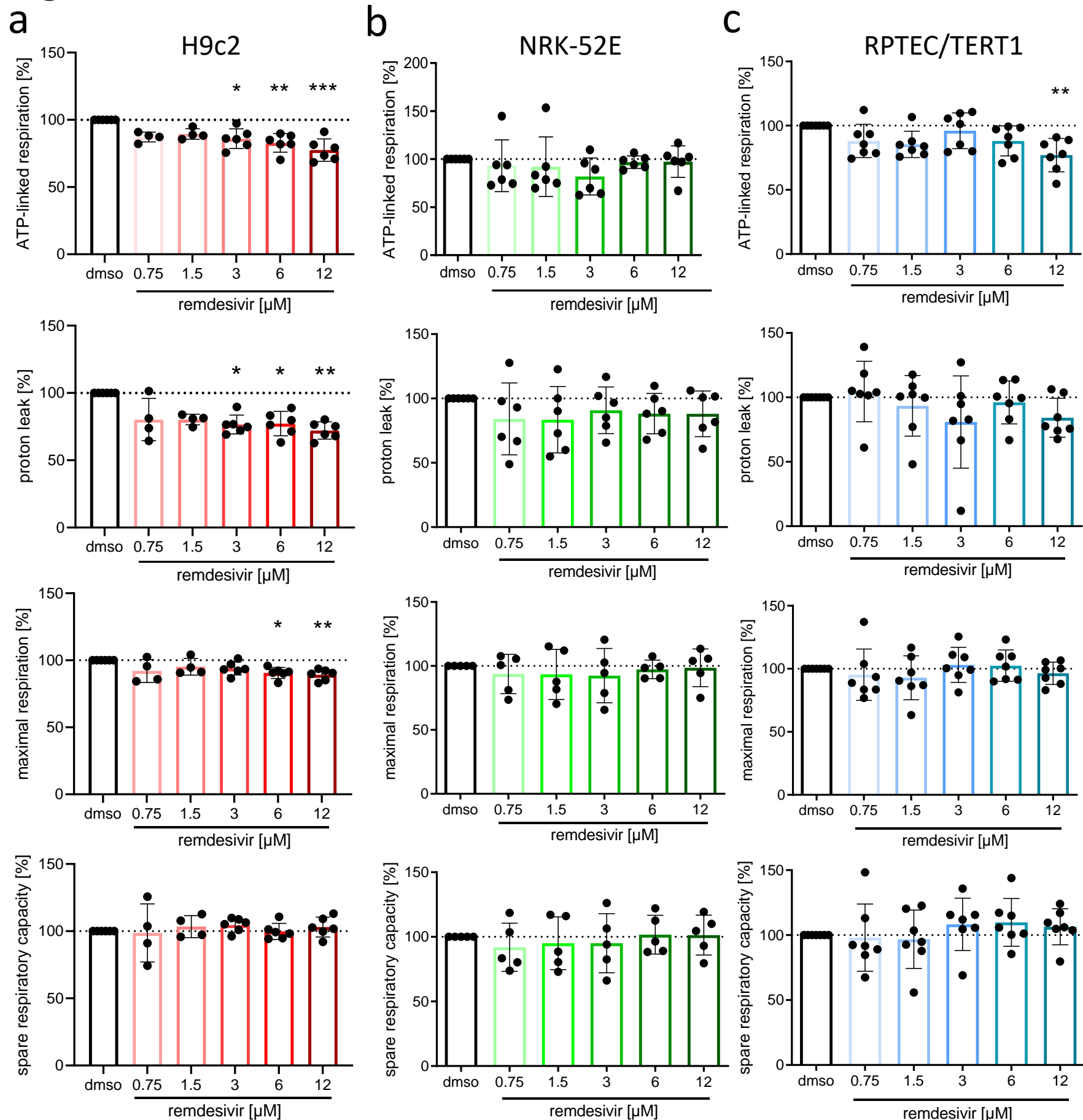


Fig. S3 The effect of remdesivir on different parameters of mitochondrial function (a) H9c2, (b) NRK-52E or (c) RPTEC/TERT1 cells were treated with increasing concentrations of remdesivir for 24 h. 1 h after remdesivir removal the oxygen consumption rate (OCR) was measured during injections of the inhibitors of the respiratory chain oligomycin, FCCP or rotenone/antimycin A. Different parameters were calculated from the OCR as described in material and methods (n = 5-6). Data were normalized to the solvent controls (dmso = 100 %; dashed line) Kruskal-Wallis-test with Dunn's multiple comparisons test * p < 0.05, ** p < 0.01, *** p < 0.001

Figure S4

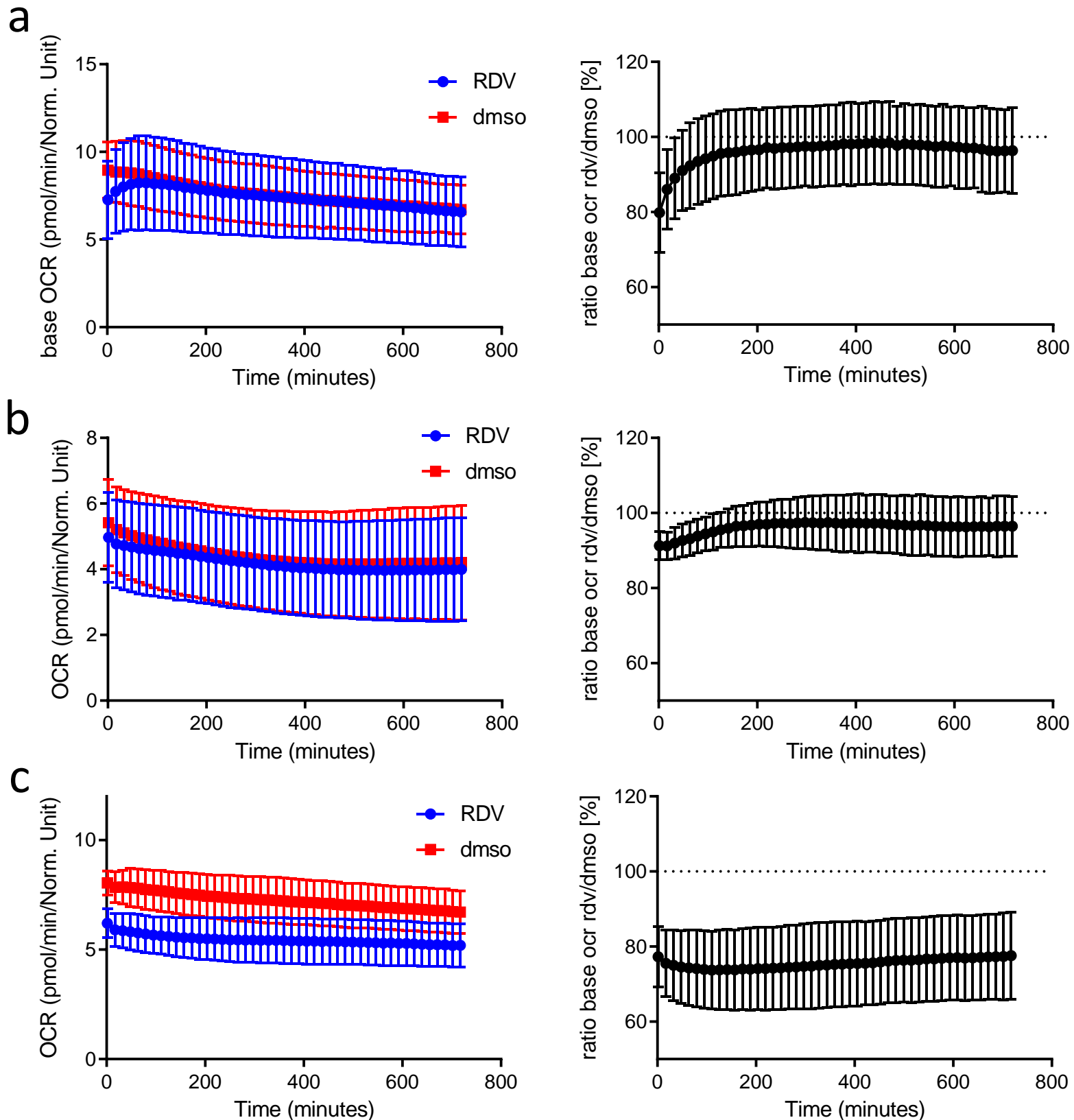


Fig. S4 Reversibility of remdesivir's effect on mitochondrial function

(a) H9c2, (b) NRK-52E or (c) RPTEC/TERT1 cells were treated with 12 μ M (a, c) or 3 μ M (b) of remdesivir for 24 h. 1 h after remdesivir removal the oxygen consumption rate (OCR) was measured every 15 min for 12 h. The OCR, which was normalized only to the cell number (left) and the OCR-ratio of remdesivir/dmsol (right) over time is shown (mean, SD, n = 6).

Figure S5

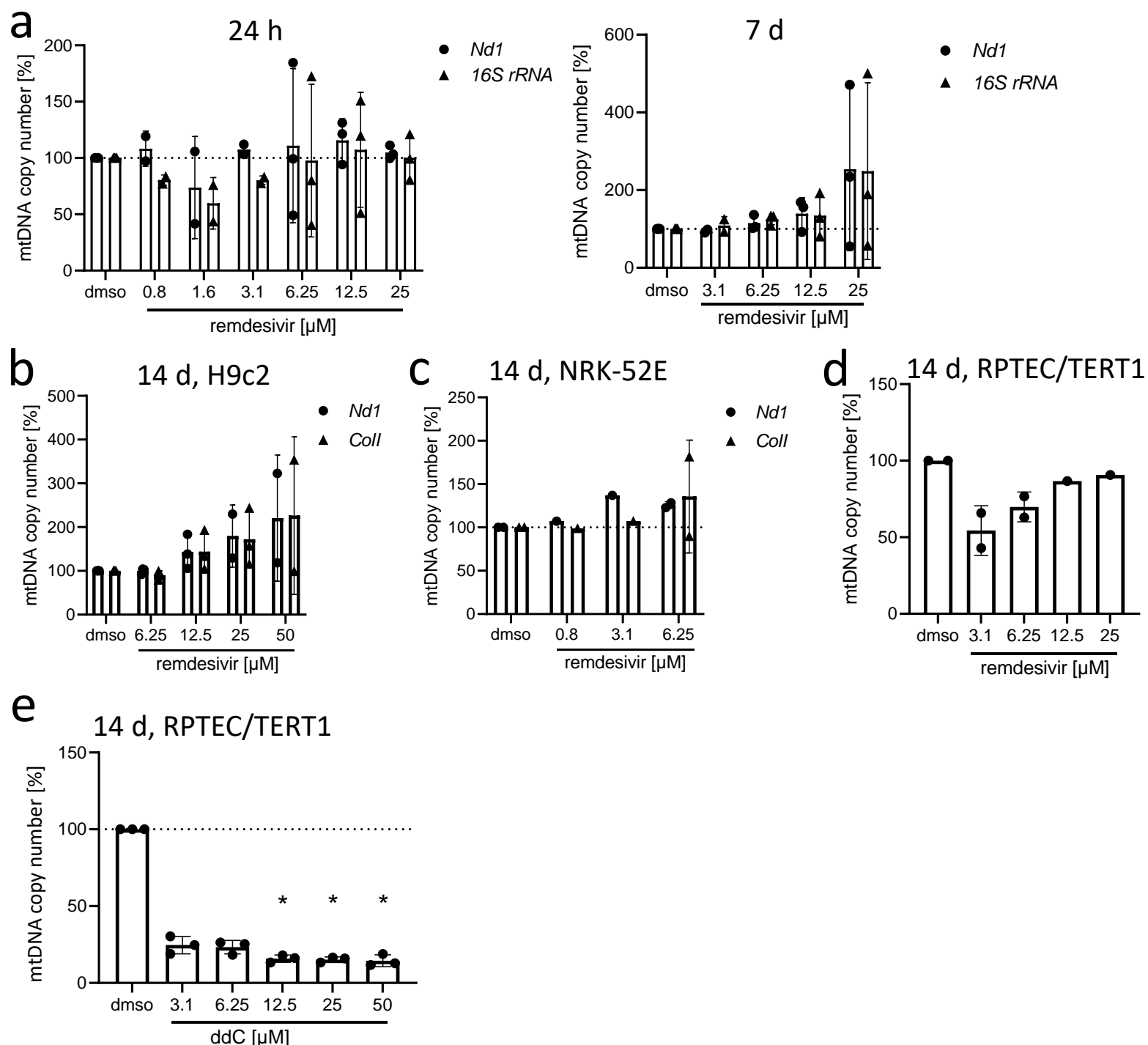


Fig. S5 Effect of remdesivir on mitochondrial DNA copy number

(a) NMCM were treated with increasing concentrations of remdesivir for 24 h (n = 2-3) or 7 days (n = 3). DNA was isolated and qPCR for mouse mitochondrial genes *Nd1* and *16S rRNA* and nuclear gene *Hk2* was performed. (b) H9c2 (n = 2-3), (c) NRK-52E (n = 2) or (d, e) RPTEC/TERT1 cells (were treated with remdesivir (b, c, d) or ddC (e; n = 3) repetetively every 3rd day for 14 days. DNA was isolated and qPCR for rat mitochondrial genes *Nd1* and *Coll* and nuclear gene *AtIII* (b, c) or for human mitochondrial DNA and the nuclear gene *B2M* (d, e) was performed. (a-e) The mitochondrial copy number was normalized to the solvent control (dmso = 100 %, dashed line) Kruskal-Wallis-test with Dunn's multiple comparisons test * p < 0.05, ** p < 0.01, *** p < 0.001

Figure S6

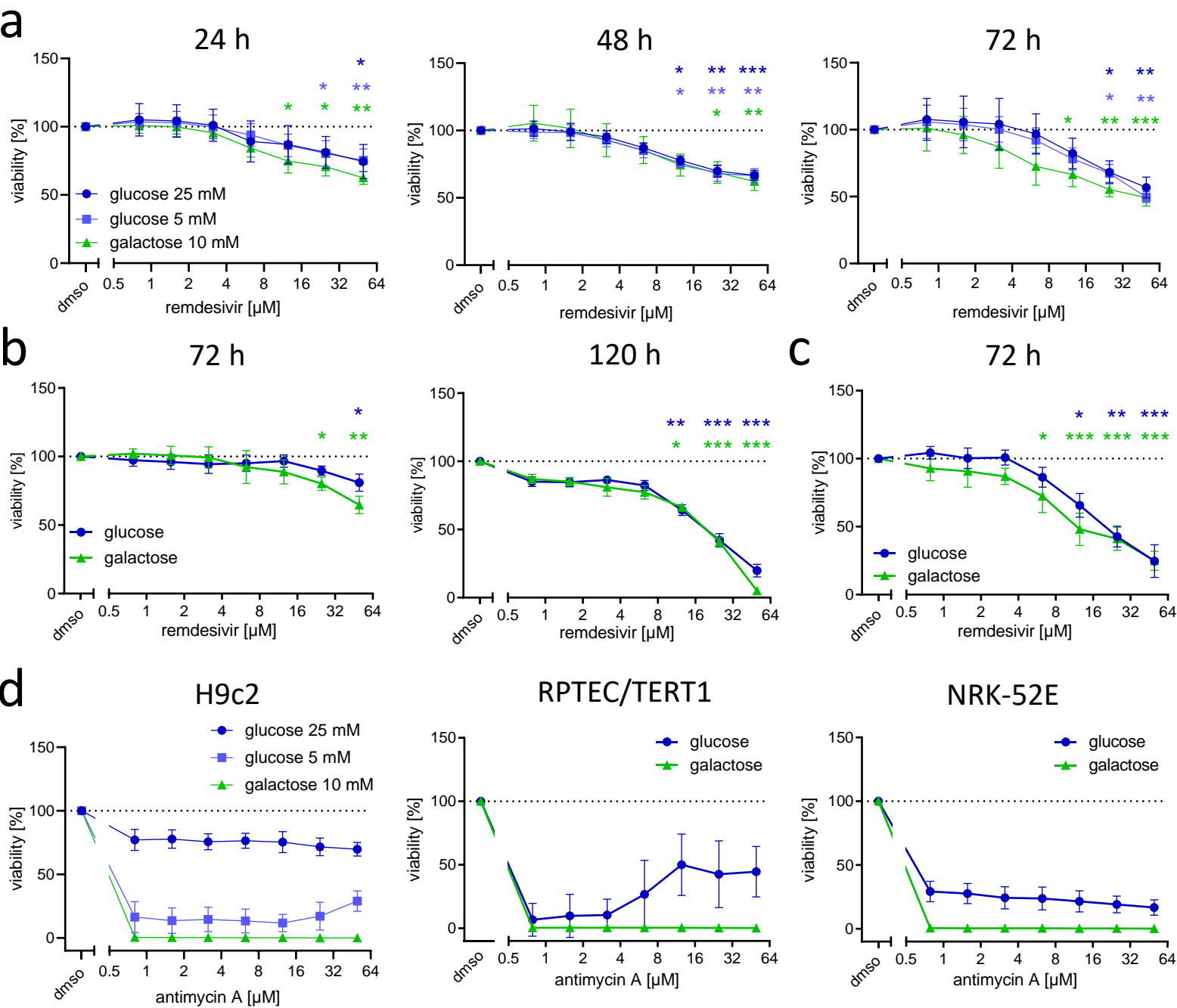


Fig. S6 The effect of different metabolic conditions on the toxicity of remdesivir and antimycin A. Cells were treated with increasing concentrations of remdesivir (**a - c**) or antimycin A (**d**) and viability was determined by ATP-measurement after indicated time periods. (**a**) H9c2 cells were cultured and treated in medium containing 25 mM glucose, 5 mM glucose or 10 mM galactose (n = 5). (**b**) RPTEC/TERT1 cells were cultured and treated in medium containing 10 mM glucose or 10 mM galactose. Within the 120h period cells were retreated once (n = 5-7). (**c**) NRK-52E cells were cultured and treated in medium containing 25 mM glucose or 10 mM galactose (n = 7). (**d**) Indicated cell lines were treated with increasing concentrations of antimycin A for 72 h (H9c2: n = 3, RPTEC: n = 7, NRK-52E: n = 6) Data were normalized to the solvent controls (dms0 = 100 %, dashed line). Kruskal-Wallis-test with Dunn's multiple comparisons test * p < 0.05, ** p < 0.01, *** p < 0.001

Figure S7

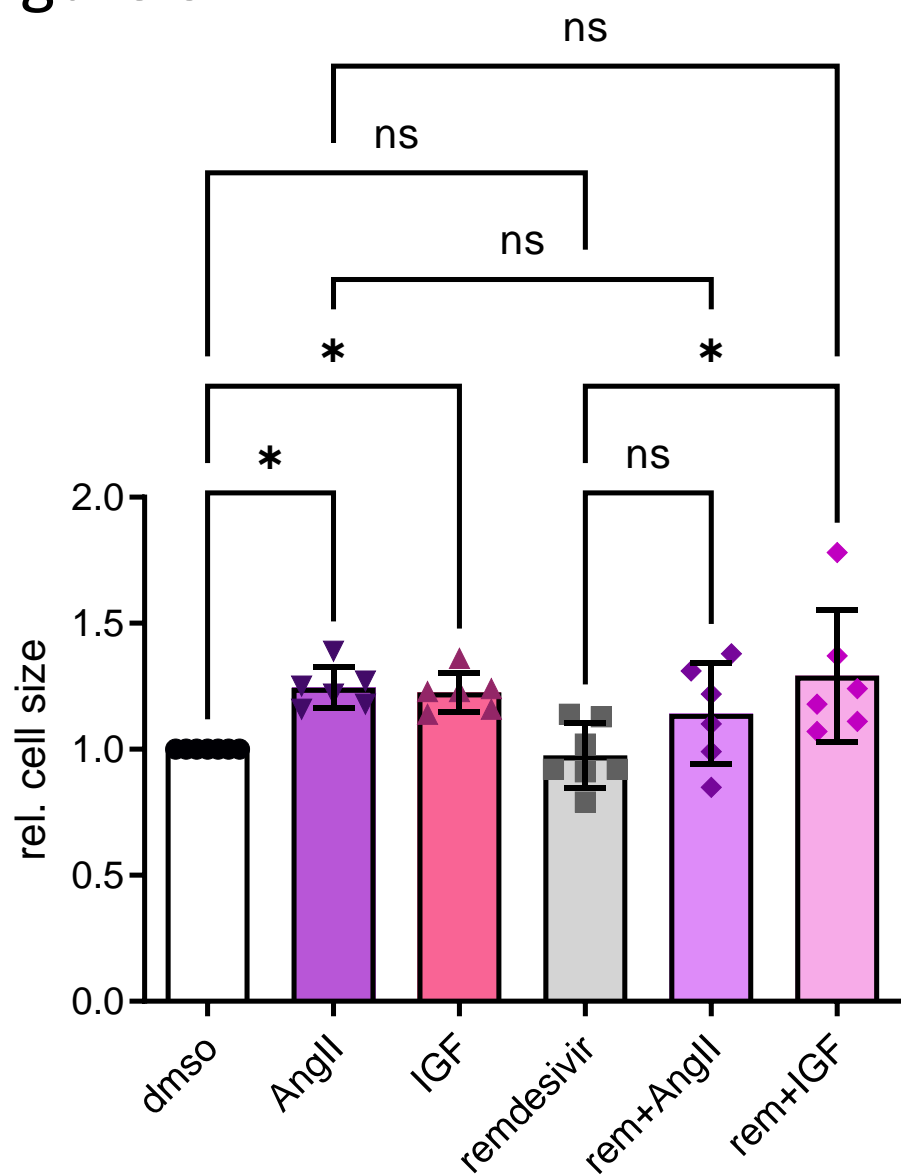


Fig. S7 Effect of remdesivir on the hypertrophic response of NMCM
NMCM were treated with 200 nM angiotensin II (AngII) or 300 pM insulin-like growth factor (IGF) with or without co-treatment with 6.25 μ M remdesivir for 24 h. Cell sizes were determined by microscopical examination and normalized to the solvent control (dms0 = 1). Kruskal-Wallis-test with Dunn's test for multiple comparisons (n = 6; * p < 0.05)

Figure S8

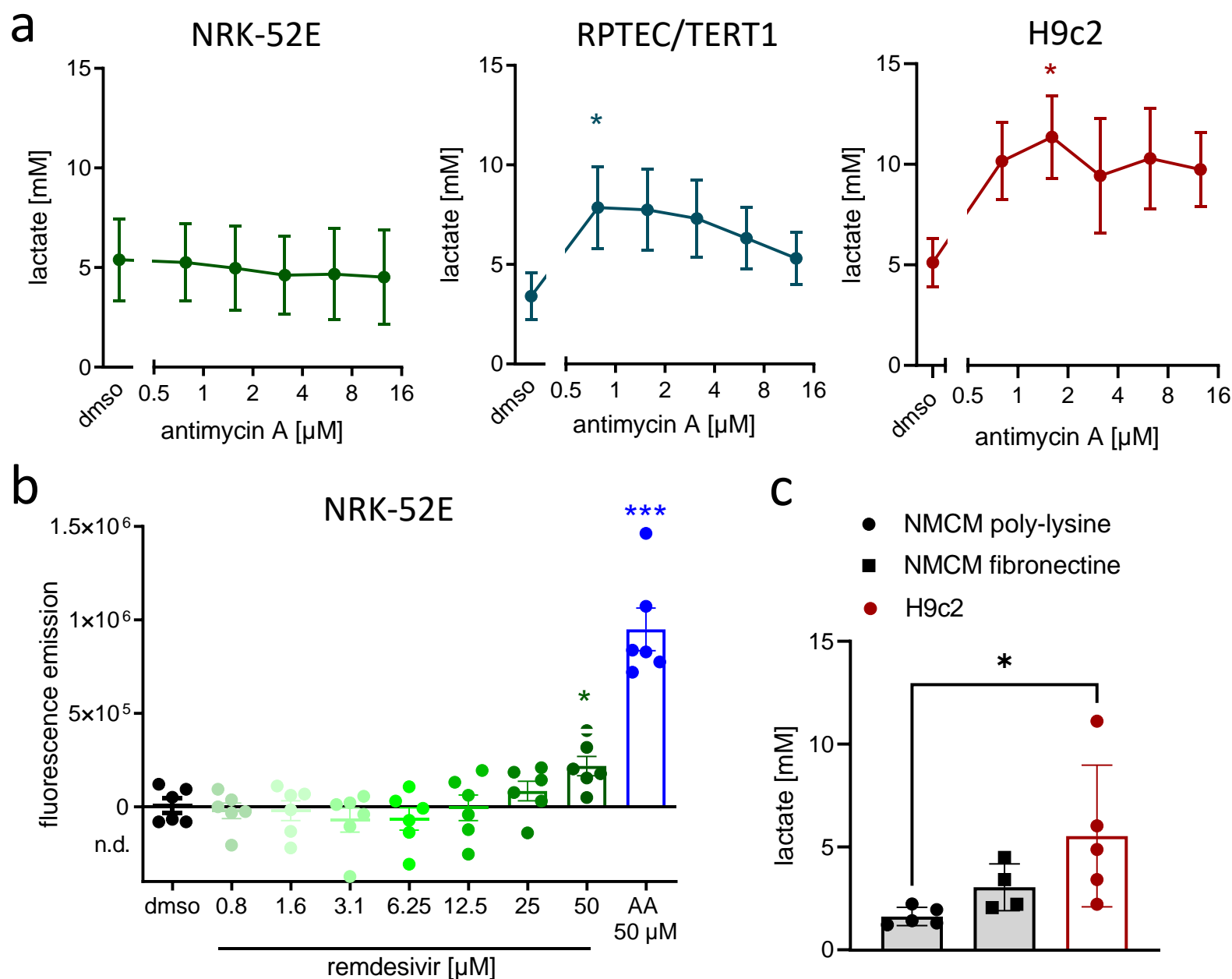


Fig. S8 Comparison of the relative sensitivity towards mitotoxic agents between different cell lines

(a) NRK-52E (n = 9), RPTEC/TERT1 (n = 7) or H9c2 cells (n = 3) were treated with increasing doses of antimycin A or solvent control (dms0) for 24 h and lactate was quantified in the supernatants. (b) NRK-52E cells were treated with remdesivir, antimycin A (AA) or solvent control (dms0) for 24 h and ROS-release was quantified in the supernatants by fluorescent detection of H_2O_2 (n = 6). (a, b) RM-ANOVA with Dunnet's multiple comparisons test * $p < 0.05$, *** $p < 0.001$ (c) indicated cell types were treated with 0.1 % dms0 for 24 h. Lactate concentrations were determined in supernatants. Data are also shown in a normalized way in Fig. 3 a (H9c2) and Fig. 3 c, e (NMCM). One-way ANOVA * $p < 0.05$